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N-Acetyl-β-D-glucosaminidase in Marmoset Kidney, Serum and Urine

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N-Acetyl-β-D-glucosaminidase activities were determined in homogenates of marmoset kidney, in serum and in urine by using the 4-methylumbelliferyl substrate. The enzyme activity was separated into several components by DEAE-cellulose ion-exchange chromatography, starch-gel electrophoresis and isoelectric focusing. The kidney contained two major forms of the enzyme, A and B, which had similar pH optima and K_m values. The A-form bound to DEAE-cellulose at pH 6.8, migrated towards the anode on starch-gel electrophoresis and had a pI of 5.0. The B-form did not bind to DEAE-cellulose at pH 6.8, remained near the origin on starch-gel electrophoresis and had a pI of 7.64. The isoenzymes also differed in heat stability, the B-form being the more stable. Serum contained B-form activity and, in addition, two intermediate forms (I_1 and I_2) were loosely bound to DEAE-cellulose. The serum A-form activity was less firmly bound to DEAE-cellulose than was the tissue A-form and was designated A^s. Serum from a pregnant marmoset contained a form which may be analogous to the human P-isoenzyme. Urine contained only a small amount of B-form activity, the majority being present in the A-form. The kidney A- and B-forms both had mol.wts. of 96000–100000 and the activity was predominantly lysosomal. Partial purification of the kidney A isoenzyme was undertaken. Immunoprecipitation studies indicated a relationship between marmoset kidney A-form and human liver A-form activity.

N-Acetyl-β-D-glucosaminidase (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) exists in two major forms in the tissues of a number of different species. The A- and B-forms were originally isolated from human spleen by Robinson & Stirling (1968). Both isoenzymes have been purified from human placenta (Geiger & Arnon, 1976) and also human kidney (Marinkovic & Marinkovic, 1977; Wiktorowicz *et al.*, 1977).

In addition to the A- and B-forms, other minor isoenzymes of *N*-acetyl-β-D-glucosaminidase have been demonstrated in human tissues. Serum contains a form of *N*-acetyl-β-D-glucosaminidase that has a higher isoelectric point than does the tissue A-form, and this isoenzyme has been designated A^s (B. G. Ellis *et al.*, 1975). In addition, serum contains two isoenzymes, I_1 and I_2 (Price & Dance, 1972), that can be separated by DEAE-cellulose chromatography, and these forms have electrophoretic mobilities intermediate between those of forms A and B. During pregnancy, another isoenzyme, P, appears in human serum, accounting for as much as 68% of the total *N*-acetyl-β-D-glucosaminidase activity at term (Stirling, 1972). This form also has an intermediate electrophoretic mobility and is eluted from DEAE-cellulose by a salt concentration similar to that which elutes the I_2-form. Another form, S, that migrates more rapidly than the tissue A-form on electrophoresis, is present in serum (Srivastava *et al.*, 1975). The residual *N*-acetyl-β-D-glucosaminidase activity in Sandhoff's disease is present as the S-isoenzyme. Another form of *N*-acetyl-β-D-glucosaminidase that migrates rapidly on electrophoresis is the C-form, present in human brain (Braidman *et al.*, 1974). This isoenzyme has a neutral pH optimum and is immunologically distinct from the A- and B-forms. The C- and S-forms can be separated by starch-gel electrophoresis (Swallow *et al.*, 1976). Another form of *N*-acetyl-β-D-glucosaminidase is present in male urine and elutes from DEAE-cellulose under high-salt conditions, and this was designated the M-form by Grebner & Tucker (1973).

The A-isoenzyme is absent in Tay–Sachs disease (Okada & O'Brien, 1969) and neither A- nor B-form activity is present in Sandhoff's disease (Sandhoff *et*

al., 1968). Interest in the prenatal diagnosis of storage diseases gave impetus to our investigation of human *N*-acetyl-β-D-glucosaminidase, and study was further stimulated by the finding that the activity of this enzyme was increased in the urine of experimental animals during acute kidney damage (Robinson *et al.*, 1967*b*; Ellis & Price, 1975). The increase of the activity of *N*-acetyl-β-D-glucosaminidase in the urine of patients with renal allografts can be used as an early warning of rejection (Wellwood *et al.*, 1973) and as an indicator of active renal disease (Dance *et al.*, 1970; Wellwood *et al.*, 1975). When pathological urine samples were subjected to starch-gel electrophoresis, it was shown that the increase of *N*-acetyl-β-D-glucosaminidase activity was accompanied by an increase in the proportion of the B-isoenzyme in the urine (Price *et al.*, 1970). The percentage of the B-form present in the urine is related to the severity of renal disease (B. G. Ellis *et al.*, 1975).

Difficulties encountered in the assessment of disease in patients have stimulated interest in the establishment of animal models for renal disease. Such a model is also required for the detection of nephrotoxicity of new drugs. The marmoset is a small primate, and its value as an animal model for the investigation of nephrotoxicity has been carried out (Pierce *et al.*, 1977*a*). A preliminary communication (Pierce *et al.*, 1975) described some of the properties of isoenzymes I and II of *N*-acetyl-β-D-glucosaminidase in marmoset kidney. In the present paper additional evidence is presented that suggests that these isoenzymes can be renamed A and B since they have many features in common with the human isoenzymes. Some of the properties of the minor forms of *N*-acetyl-β-D-glucosaminidase in the marmoset are described and the similarities between these forms and those found in human tissue are discussed.

Materials and Methods

Tissues and fluids

Kidneys were removed from common marmosets (*Callithrix jacchus*, bred at ICI Pharmaceuticals, Alderley Park, Cheshire, U.K.) killed under diethyl ether anaesthesia and either used immediately for subcellular studies or frozen at −20°C. Homogenates were prepared from frozen tissue as described by Pierce *et al.* (1977*b*). Blood was taken from the femoral vein in quantities up to 1 ml and left to clot for 1 h before centrifugation at 1500*g* for 15 min to separate the serum. Urine was collected on ice from marmosets maintained overnight (16 h) in metabolism cages with water *ad libitum*, but without food, and the urine was stored at 4°C for subsequent investigations.

Subcellular fractionations

Subcellular fractions were prepared from kidney homogenates as described by Pierce *et al.* (1977*b*). Acid phosphatase was assayed as described by Price & Dance (1967). The latency of enzyme activities in the subcellular fractions was assayed at 25°C for 10 min with substrate solutions in 0.45 M-sucrose for estimations of free activity, and in 0.45 M-sucrose plus 0.1% Triton X-100 for assaying the total activity (Price & Dance, 1967).

Enzyme assays

N-Acetyl-β-D-glucosaminidase activity was assayed by the method of Robinson *et al.* (1967*a*). Alternatively, a modification of the automated assay developed by Tucker *et al.* (1975) was used.

Gel filtration

Gel filtration was performed either with Sephadex G-200 [fine grade; Pharmacia (G.B.), London W.5, U.K.] as described by B. G. Ellis *et al.* (1975), or with Bio-Gel P-200 (Bio-Rad Laboratories, CA, U.S.A.) equilibrated in 10 mM-sodium phosphate buffer, pH 6.0, in a column (40 cm × 2.5 cm diam.). Loading and elution of enzyme samples (0.5 ml) was carried out on Bio-Gel P-200 at 4°C with a flow rate of 15 ml/h, and fractions (5 ml) were collected.

Ion-exchange chromatography

DEAE-cellulose (Whatman DE 52; Whatman, Maidstone, Kent, U.K.) was equilibrated with 10 mM-sodium phosphate buffer, pH 6.8, the separation was carried out by the method of R. B. Ellis *et al.* (1975). The column contained 1 ml of DEAE-cellulose and the eluate was passed into an autoanalytical system to give a continuous trace of enzyme elution.

Neuraminidase treatment of marmoset serum

The effects of neuraminidase [type VI from *Clostridium perfringens*; Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K.] on the serum *N*-acetyl-β-D-glucosaminidase isoenzymes were investigated as follows. Serum (50 μl) was incubated with 10 mM-sodium phosphate buffer, pH 5.0 (50 μl), and neuraminidase (50 μl of a 1 unit/ml solution in 10 mM-sodium phosphate buffer, pH 5.0) at 37°C for 3 h.

Isoelectric focusing

Focusing of sample was carried out in the pH ranges 3.5–10 and 4–6 by using the column technique of Vesterberg & Svensson (1966) as described by Pierce *et al.* (1975).

Starch-gel electrophoresis

Gels were prepared from hydrolysed starch (Connaught Medical Research Laboratories, Toronto, Canada), and conditions of electrophoresis and detection of enzyme activity were as described by Robinson *et al.* (1967*a*).

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel disc electrophoresis was performed as described by Davis (1964). Enzyme activity was detected by cutting the gel into 2mm slices and each slice was disrupted by homogenization. The homogenized slice was incubated with 0.5ml of 1mM-4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside in 0.1M-Na_2HPO_4/0.5M-citric acid buffer, pH 4.5, at 37°C for 1h; 0.5M-glycine/NaOH buffer, pH 10.4 (1.5ml), was then added to stop the reaction, and the fluorescence was read as described by Robinson *et al.* (1967*a*).

Purification of N-acetyl-β-D-glucosaminidase from marmoset kidney

A homogenate (10%, w/v) was prepared from 20*g* of marmoset kidneys in 10mM-sodium phosphate buffer, pH 6.8, with a Waring blender for 5min at 4°C. $(NH_4)_2SO_4$ was then added to bring the homogenate to 30% saturation, and the mixture was stirred for 2h at 4°C before centrifugation at 12000*g* for 30 min. The resulting supernatant was then brought to 60% saturation by the addition of solid $(NH_4)_2SO_4$ and was stirred at 4°C overnight. The precipitate was separated by centrifugation as before and redissolved in the minimum quantity of 10mM-sodium phosphate buffer (this buffer was used throughout the purification). The solution was dialysed against several changes of buffer until sulphate could no longer be detected in the diffusate. The dialysed kidney extract was gently stirred for 1h at 4°C with concanavalin A–Sepharose (Pharmacia; 10ml) that had been previously washed with 400ml of buffer containing 0.5M-NaCl. The gel was packed into a 50ml syringe barrel fitted with a porous plastic sinter (Vyon; Portland Plastics, Hythe, Kent, U.K.) and was washed with buffer containing 0.5M-NaCl and the A_{280} of the elute was monitored. Washing was continued until the A_{280} was maintained near zero. Adsorbed glycoproteins were eluted with 0.25M-methyl α-glucoside in buffer. This eluate was concentrated against buffer by vacuum dialysis at 4°C as described by Huehns & Shooter (1961). The concentrated eluate was applied to a column (80cm×5cm diam.) containing Sephadex G-75 and eluted with buffer under gravity at 4°C. Fractions containing *N*-acetyl-β-D-glucosaminidase activity were pooled and concentrated against buffer by vacuum dialysis at 4°C. The concentrated eluate was applied to a column (80cm×2.5cm diam.) containing DEAE-cellulose equilibrated in buffer at 4°C. The column was washed with buffer until no further protein was eluted (measured by the A_{280}) and the activity retained on the column was then eluted with a linear salt gradient (0–0.5M-NaCl in 800ml of buffer).

The affinity ligand 2-acetamido-1-*N*-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine was prepared and coupled to Sepharose 4B (Pharmacia) as described by Koshy *et al.*, (1975) and was a gift from Dr. T. Bearpark. The ligand coupled to Sepharose 4B (1ml) was packed into a 2ml disposable-syringe barrel containing a Vyon sinter and washed with 20ml of buffer. The retained activity from the DEAE-cellulose column was concentrated by vacuum dialysis against buffer, further dialysed against buffer and then applied to the column. The column was then washed successively with buffer (20ml), 40mM-*N*-acetyl-β-D-glucosamine in buffer (10ml), 30mM-NaCl in buffer (10ml), 30mM-NaCl plus 40mM-*N*-acetyl-β-D-glucosamine in buffer (10ml), and finally 1M-NaCl in buffer (20ml) to remove any remaining protein.

Immunoprecipitation

Antiserum to purified *N*-acetyl-β-D-glucosaminidase A from human liver raised in rabbits was a gift from Dr. T. Bearpark. Antiserum of the required dilution (10μl) was incubated with 50μl of the marmoset hexosaminidase A preparation at room temperature for 2h. The mixture was then centrifuged at 1500*g* for 15min in a 16×15ml swing-out rotor (r_{av}. 7.5cm) in an MSE Super Minor centrifuge. The supernatant (20μl) was then assayed for enzyme activity as described by Robinson *et al.* (1967*a*).

Results

Enzyme kinetics of marmoset N-acetyl-β-D-glucosaminidase

Some kinetic characteristics of *N*-acetyl-β-D-glucosaminidase in marmoset kidney, serum and urine are shown in Table 1. The kidney homogenate activity and the isoenzymes A and B from the kidney separated by DEAE-cellulose ion-exchange chromatography had a wide pH optimum. The enzyme in serum and urine, however, had a single sharp pH optimum at pH 4.6 and 4.5 respectively. Subsequent assays were performed at pH 4.5 in all cases.

The Michaelis constants for the enzyme from different sources show small differences, but these are not regarded as significant. The stability of the enzyme from serum, urine and kidney to heat is shown in Table 1 and in Fig. 1. The kidney homo-

Table 1. *Kinetic characteristics of N-acetyl-β-D-glucosaminidase in marmoset kidney, serum and urine*
Enzyme assays were performed as described in the Materials and Methods section. The percentage of each isoenzyme was determined from the elution profiles after DEAE-cellulose chromatography. pH optima were determined as described by Pierce *et al.* (1977*b*) in sodium phosphate/citric acid buffer. Heat-stability values are the percentages of activity remaining after 2h at 50°C.

	Enzyme activity	Isoenzyme composition (%) A	B	pH optimum	K_m (mM)	Heat stability (%)
Kidney homogenate	86 ± 8.7(7)*	85–90	10–15	4.2–4.8	0.70	26
Kidney A-form	—	—	—	4.5–4.7	0.64	7
Kidney B-form	—	—	—	4.5–4.8	0.64	49
Urine	7.2 ± 3.0 (26)† 187 ±87 (26)‡	90–97	3–10	4.5	0.74	7
Serum	0.49± 0.4 (4)§	50–65	10–35	4.6	0.56	35

* μmol/h per g wet wt. of tissue.
† nmol/h per mg of creatinine.
‡ nmol/h per 16h sample.
§ μmol/h per ml.

genate activity showed a biphasic response to incubation at 50°C (Fig. 1*a*) as did the serum activity (Fig. 1*a*); 74% of the kidney homogenate activity was lost after 2h, but the remaining activity was stable for a further 2h. When the separated isoenzymes were incubated at 50°C, 93.5% of the activity of form A was lost after 2h, but only 51% of the B-form activity was lost (Fig. 1*b*). The activity of the enzyme in urine followed the pattern for kidney form A; 94% was lost after 2h at 50°C (Fig. 1*a*).

The pH-stability of the kidney *N*-acetyl-β-D-glucosaminidase was tested by incubation for 30min at 37°C at the required pH before assaying the activity at pH 4.5. The enzyme was stable between pH 4 and 6, but activity was lost outside this range. At pH 2.2 all activity was lost, and at pH 8.9 only 33% remained.

Inhibition of kidney N-acetyl-β-D-glucosaminidase

N-Acetyl-β-D-glucosamine and *N*-acetyl-β-D-galactosamine competitively inhibited marmoset kidney *N*-acetyl-β-D-glucosaminidase. *N*-Acetyl-β-D-glactosamine was the more potent inhibitor (K_i 0.08) than *N*-acetyl-β-D-glucosamine (K_i 1.22). When the enzyme was assayed in the presence of 1.0mM-*N*-acetylgalactosamine, 75% of the activity was lost, but fell by only 26% when 1.0mM-*N*-acetyl-β-D-glucosamine was used.

Marmoset kidney *N*-acetyl-β-D-glucosaminidase was inhibited in 0.1M-sodium acetate/acetic acid buffer. A pH gradient constructed with this buffer gave an apparent pH optimum at pH 5.6, but at this pH only 20% of the activity in 0.1M-sodium phosphate/citric acid buffer was found.

Subcellular distribution of N-acetyl-β-D-glucosaminidase in the kidney

The distribution of *N*-acetyl-β-D-glucosaminidase in the marmoset kidney is shown in Fig. 2 and is compared with the distribution of the lysosomal marker enzyme acid phosphatase. The *N*-acetyl-β-D-glucosaminidase activity is predominantly in the lysosomal/mitochondrial fraction, whether the activity is expressed as specific activity (Fig. 2*a*) or as relative specific activity (Fig. 2*b*). Acid phosphatase is predominantly lysosomal when the activity is assessed as specific activity, although there is significant activity in the mitochondrial-microsomal and microsomal fractions. When the acid phosphatase activity is expressed as relative specific activity the activity in the microsomal fraction is emphasized. It is possible that acid phosphatase is not a particularly good lysosomal marker enzyme for the marmoset kidney, or alternatively the substrate used is not specific for the lysosomal activity.

Gel filtration

A single peak of *N*-acetyl-β-D-glucosaminidase activity was eluted when the kidney homogenate was subjected to gel filtration on Sephadex G-200. This peak corresponded to an apparent mol.wt. of 96000 ($K_{av.}$ 0.28 or V_e/V_0 1.66). When gel filtration was carried out with Bio-Gel P-200, a mol.wt. of 100000 was obtained for the kidney enzyme, which was again eluted as a single peak. The *N*-acetyl-β-D-glucosaminidase activity of urine was also eluted from Bio-Gel P-200 immediately after the void volume and had an apparent mol.wt. of 112000.

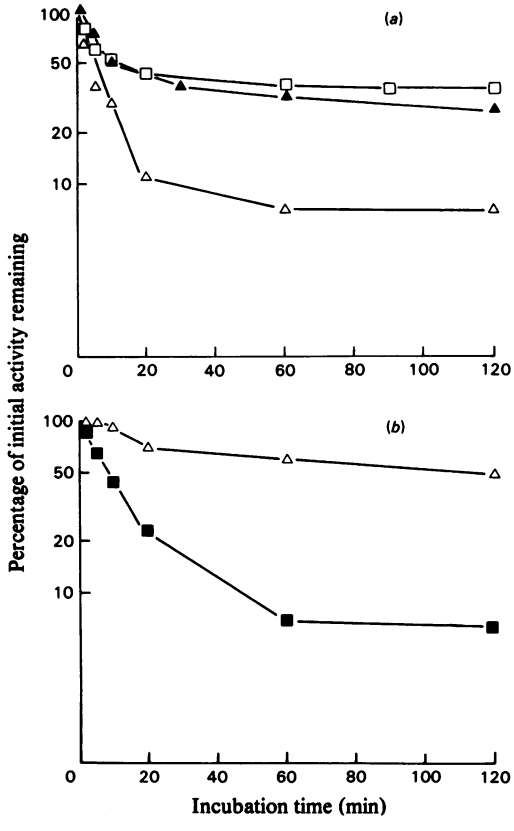

Fig. 1. *Heat-stability of N-acetyl-β-D-glucosaminidase in marmoset kidney, serum and urine*

Diluted enzyme preparations were incubated at 50°C in 10mM-sodium phosphate buffer, pH 6, and samples (0.1ml) were removed at different times and assayed under standard conditions. (*a*) Kidney homogenate (▲), serum (□) and urine (△) *N*-acetyl-β-D-glucosaminidase; (*b*) marmoset kidney *N*-acetyl-β-D-glucosaminidase isoenzyme A (■) and B (△).

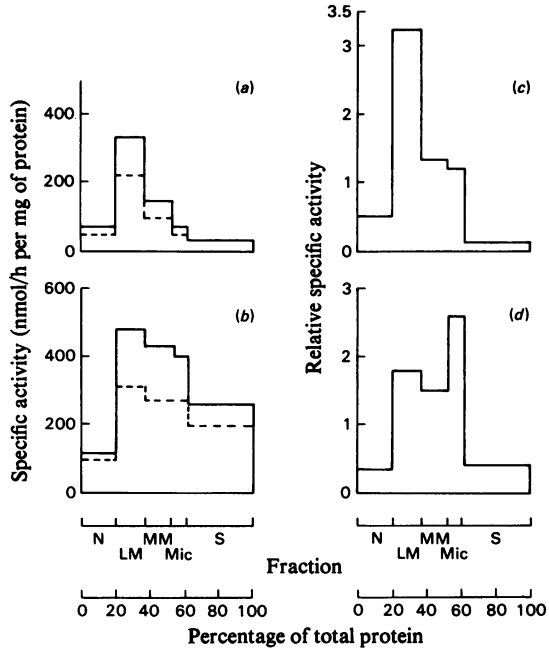

Fig. 2. *Subcellular distribution of marmoset kidney N-acetyl-β-D-glucosaminidase and acid phosphatase*

Specific activities are given as nmol/h per mg of protein and relative specific activities as the percentage of the total specific activity in each fraction divided by the percentage of the total protein in that fraction. The abscissae give the percentages of total protein in each fraction in order of elution: N, nuclear fraction; LM, lysosomal–mitochondrial fraction; MM, mitochondrial–microsomal fraction; Mic, microsomal fraction; S, soluble fraction. Enzymes were assayed as described in the text: ——, total activity; ----, free activity. (*a*) *N*-Acetyl-β-D-glucosaminidase, specific activity; (*b*) acid phosphatase, specific activity; (*c*) *N*-acetyl-β-D-glucosaminidase, relative specific activity; (*d*) acid phosphatase, relative specific activity.

DEAE-cellulose ion-exchange chromatography

The semi-automated ion-exchange technique of R. B. Ellis *et al.* (1975) gives a continuous elution profile and is sensitive to minor changes in isoenzyme pattern. The elution profiles of a kidney supernatant, serum and urine prepared from marmosets are compared in Fig. 3. A small proportion (11%) of the recovered activity failed to bind to the column (B-form) when the kidney supernatant was applied to DEAE-cellulose, pH 6.8 (Fig. 3*a*), a small intermediate peak, I_2 (6%), was displaced at a low salt concentration, but the major proportion of the enzyme activity (A-form, 76%) was eluted by higher salt concentrations. A small peak (8%) of activity was consistently observed to elute after the A-form peak. The urine profile (Fig. 3*b*) is similar to that for the kidney and the A-form peak (97%) eluted at the same salt concentration as were the kidney isoenzymes. A small amount of unretained activity (3%) was present, and in some samples an intermediate peak was also present. Serum (Fig. 3*c*) contained a higher proportion (22%) of unretained activity than the marmoset kidney urine and a larger amount of intermediate activity (7–35%) eluted in the same region as the human serum I_1- and I_2-forms (Price & Dance, 1972). The major peak in serum (45–60%) was eluted at a lower salt concentration than were the main kidney and urine isoenzymes. Serum from a pregnant marmoset

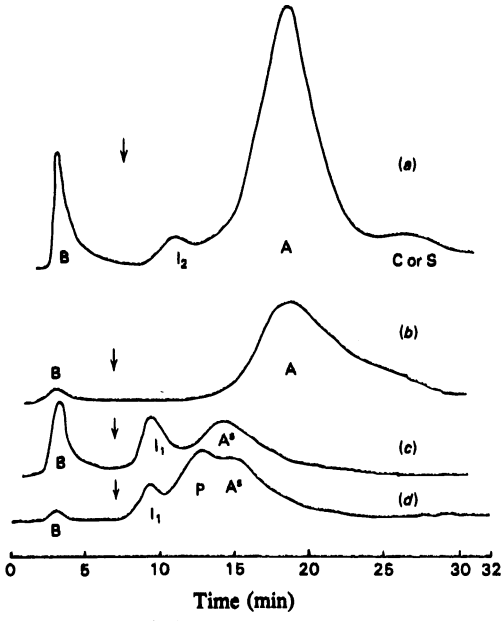

Fig. 3. *DEAE-cellulose elution profiles of N-acetyl-β-D-glucosaminidase in kidney, serum, urine and pregnancy serum*

DEAE-cellulose micro-column eluates were pumped directly into an autoanalyser system to give a continuous elution profile of: (*a*) marmoset kidney homogenate; (*b*) marmoset urine; (*c*) serum from male marmoset; (*d*) serum from a pregnant marmoset in the sixth week of 21-week gestation. The arrow represents the start of the linear NaCl gradient.

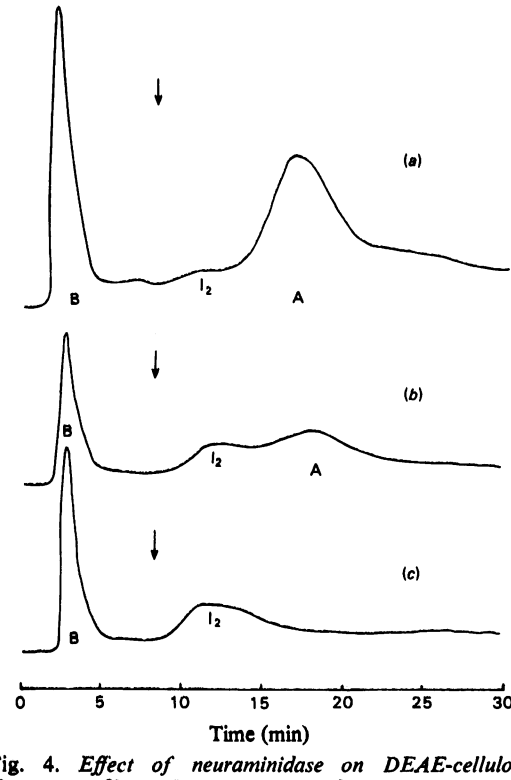

Fig. 4. *Effect of neuraminidase on DEAE-cellulose elution profiles of isoenzymes of marmoset serum N-acetyl-β-D-glucosaminidase*

Neuraminidase treatment was carried out at 37°C for 3h as described in the text. (*a*) Untreated serum; (*b*) untreated serum after incubation for 3h at 37°C; (*c*) serum treated with neuraminidase for 3h at 37°C.

showed an increase in the proportion of intermediate activity (Fig. 3*d*); the elution profile of this form is analogous to that of the P-form described in serum from pregnant women (Stirling, 1972).

The effects of neuraminidase treatment on serum *N*-acetyl-β-D-glucosaminidase isoenzymes are shown in Fig. 4. The elution profile of the serum sample used for preincubation, which contained a large proportion of unretained activity (37%), a small amount of intermediate material (7%) and a large retained peak (56%) is shown in Fig. 4(*a*). A sample of this serum, after incubation at 37°C for 3h at pH 5, showed a change in pattern (Fig. 4*b*). The proportion of the major retained peak had decreased to 42% of the recovered activity, and there was an increase in the intermediate peak (25%). In the neuraminidase-treated sample, all the activity was present as the intermediate (49%) and unretained forms (51%). This suggests that some interconversion of isoenzymes of *N*-acetyl-β-D-glucosaminidase can occur on incubation at 37°C and that the trend of these changes is accelerated by the addition of neuraminidase. There was no merthiolate in the neuraminidase preparation used in this experiment, since this has been shown to affect the conversion of human tissue A-form into the B-form (Carmody & Rattazzi, 1974).

Since there are striking similarities between the elution profile of *N*-acetyl-β-D-glucosaminidase from marmoset kidney, serum and urine and the human isoenzyme profiles, the use of the terms A, B, I_1 and I_2 to describe the main isoenzyme forms is justified. Form B is the unretained activity on DEAE-cellulose at pH 7.0; forms I_1 and I_2 form the intermediate peaks, and form A is the major retained peak in tissue and urine, whereas form A^S is the major retained form in normal serum.

Starch-gel electrophoresis

Marmoset kidney and serum *N*-acetyl-β-D-glucosaminidase gave two bands of activity that separated

on starch-gel electrophoresis: one band remained near the origin (B-form) and a second major band migrated towards the anode (A-form). When urine was subjected to starch-gel electrophoresis a single band of *N*-acetyl-β-D-glucosaminidase activity with the same mobility as the major kidney band was observed. When the peak fractions from DEAE-cellulose chromatography of marmoset kidney *N*-acetyl-β-D-glucosaminidase were concentrated and subjected to electrophoresis, the A-form peak corresponded to the fast-moving anodic band, and the B-form peak activity remained near the origin. It was difficult to resolve the intermediate isoenzymes in marmoset samples by using this technique.

Isoelectric focusing

Focusing of the kidney homogenate in the pH range 8.5–10 (Pierce *et al.*, 1975) gave two peaks of activity (Fig. 5*a*). The minor peak (16% of the recovered activity) formed at a pI of 7.64, and the major peak (81%) was eluted at a pI of 5.00 and had a shoulder of activity at pH 5.28. An attempt was made to resolve further the major peak of activity by focusing in the range pH 4–6 (Fig. 5*b*). A single peak was again present with a pI of 5.12, but the peak was not symmetrical and had pronounced shoulders of activity at pI values of 5.27 and 5.60.

Partial purification

The partial purification of *N*-acetyl-β-D-glucosaminidase from marmoset kidney was undertaken to investigate the relationship between the human and marmoset enzymes. The purification is shown in Table 2. Almost all the activity bound to concanavalin A-Sepharose, and 92% was recovered on elution with methyl α-glucoside (0.25M), indicating that the enzyme is possibly a glycoprotein. The conditions that had been previously used for the affinity chromatography of human liver A-form (T. Bearpark, personal communication) were used, but they were found to be less successful for purification of the marmoset enzyme. Of the applied activity, 89% bound to the glucosylamine column, but the bulk of the enzyme activity was eluted in 30mM-NaCl along with most of the remaining protein, and only a 2-fold purification was achieved at this step. Polyacrylamide-gel electrophoresis showed that one

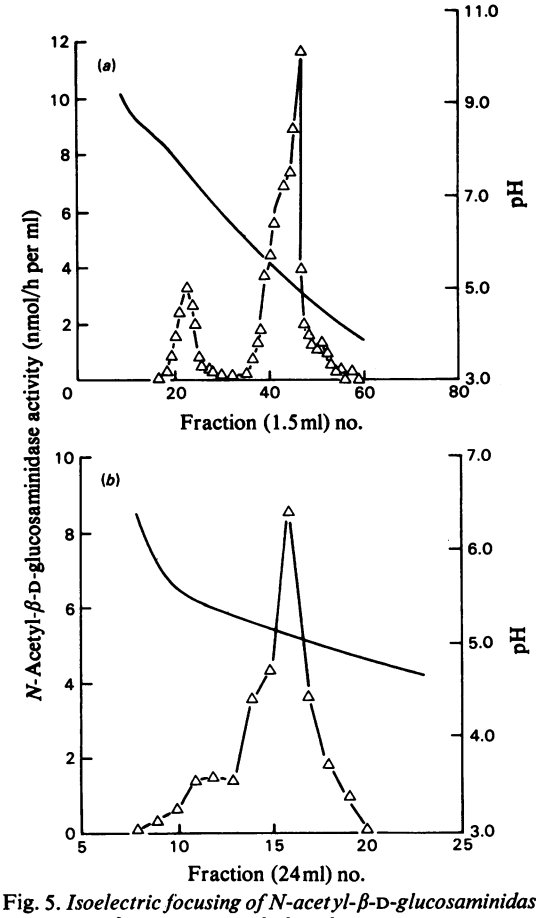

Fig. 5. *Isoelectric focusing of N-acetyl-β-D-glucosaminidase from marmoset kidney homogenate*

Isoelectric focusing was carried out as described in the text. (*a*) Focusing in the range pH 3.5–10; (*b*) focusing in the range of pH 4–6. △, *N*-Acetyl-β-D-glucosaminidase activity; ——, pH.

Table 2. *Partial purification of marmoset kidney N-acetyl-β-D-glucosaminidase A*

Fraction	Activity (μmol/h per ml)	Total activity (μmol/h)	Protein (mg/ml)	Specific activity (μmol/h per mg of protein)	Purification (fold)	Yield (%)
30%-$(NH_4)_2SO_4$ supernatant	2.9	670.0	4.750	0.6	—	100.0
Concanavalin A eluate	3.5	620.0	0.214	16.4	27.0	92.0
Sephadex G-75	3.3	88.0	0.050	64.4	107.0	73.2
DEAE-cellulose DE-52	0.8	80.0	0.010	77.0	127.0	12.0
Affinity chromatography	2.1	12.2	0.015	139.0	231.0	1.8

band of enzyme activity was recovered corresponding to the faster-moving of two bands in the kidney homogenate.

Immunoprecipitation with rabbit anti-(human liver isoenzyme A) antiserum gave a maximum precipitation of 98% of the purified marmoset *N*-acetyl-β-D-glucosaminidase A at an antiserum dilution of 100-fold.

Discussion

The major isoenzymes of *N*-acetyl-β-D-glucosaminidase described in human tissues and body fluids have their counterparts in the marmoset. The kidney A- and B-forms were separable by starch-gel electrophoresis, and the serum A^s-form was also distinguishable by this technique. DEAE-cellulose microcolumn chromatography gives a greater degree of resolution of intermediate activity, and all the isoenzymes including forms I_1, I_2, P and possibly S or C were demonstrated. The major isoenzyme (A) in urine had identical electrophoretic mobility and eluted from DEAE-cellulose at the same NaCl concentration as did the kidney A-form. However, the serum A^s-form was eluted from DEAE-cellulose by a lower NaCl concentration. The elution profile of serum *N*-acetyl-β-D-glucosaminidase was altered by both heat and neuraminidase treatment: the relative activity of the A^s-form decreased, whereas the activity of the B- and I-forms appeared to increase. A similar interconversion has also been described for the human serum A^s isoenzyme by Swallow *et al.* (1974), with starch-gel electrophoresis, and suggests that the A^s-form may be a modified form of B- or I-form activity. However, this possibility is difficult to reconcile with the absence of the A^s-form in Tay–Sachs disease (Okada & O'Brien, 1969), which suggests that A^s-form is genetically related to the tissue A-form.

The kinetic properties of the enzymes and isoenzymes in marmoset kidney serum and urine appear similar to those of the human enzymes. The Michaelis constants and pH optima for the 4-methylumbelliferyl substrate are almost identical with those for the enzyme isolated from the human spleen (Robinson & Stirling, 1968). The relative heat-stability of the isoenzymes is the same as that found for the human kidney isoenzymes (Dance *et al.*, 1969). The marmoset kidney B-form is relatively stable to treatment at 50°C, whereas the A-form is more labile. The activity from urine is also labile, reflecting the predominance of the A-form, whereas serum contains a greater proportion of heat-stable activity. Although the complement of isoenzymes in marmoset tissues and fluids resembles that found in the human, the isoenzymes from the two species do not have identical properties. The human kidney A-form is eluted from DEAE-cellulose at a higher NaCl concentration, and migrates faster than the marmoset A-form on starch-gel electrophoresis (Pierce *et al.*, 1975). The human A^s-form is also eluted from DEAE-cellulose by a higher NaCl concentration than is the marmoset isoenzyme. The marmoset kidney A and B isoenzymes both had pH optima between pH 4.2 and 4.8, and there was no evidence for a dual pH optimum as described for the human kidney isoenzymes by Marinkovic & Marinkovic (1977).

Partial purification of the marmoset kidney A isoenzyme showed that it is probably a glycoprotein, but elution from the affinity column showed differences from the properties of human liver isoenzyme A, and no specific elution of marmoset kidney isoenzyme A was achieved. However, the fact that marmoset kidney isoenzyme A is precipitated by anti-(human liver isoenzyme A) antiserum indicates that the proteins are related. The molecular weight obtained for the marmoset kidney enzyme on Sephadex G-200 (96000) and Bio-Gel P-200 (100000) is similar to the value obtained for the human kidney enzyme on Sephadex G-200 (110000) by Marinkovic & Marinkovic (1977). However, Wiktorowicz *et al.* (1977) found a higher mol.wt. (140000) for the human kidney enzyme, and they also used Sephadex G-200. The value of 140000 agrees with the findings of Lee & Yoshida (1976) for the human placental enzyme by polyacrylamide-gel electrophoresis, and with Srivastava *et al.* (1974) for the human placental enzyme on Sephadex G-200. However, Srivastava *et al.* (1974), Wiktorowicz *et al.* (1977) and Geiger & Arnon (1976) all obtained a value in the range 100000–118000 for the mol.wt. of this enzyme when measured by ultracentrifugation techniques.

The marmoset kidney, serum and urine *N*-acetyl-β-D-glucosaminidases have properties very similar to those of the human enzymes, although there are slight differences. The presence of the A, B, I_1, I_2, A^s, P, S or C isoenzymes, a similar complement to those occuring in the human, suggests that these isoenzymes have a similar function in both species. It should be noted, however, that some of these forms may not be true isoenzymes since the removal of sialic acid causes the interconversion of one form to another (Fig. 4). The similarities of the marmoset and human *N*-acetyl-β-D-glucosaminidase isoenzymes indicate that the marmoset may provide a useful model for the studies of human disease.

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